Attorney Docket No.: 10038.200-US PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Svendsen et al. Serial No.: To Be Assigned

Confirmation No: To Be Assigned Group Art Unit: To Be Assigned

Filed: June 1, 2001 Examiner: To Be Assigned

For: Cutinase Variants

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, DC 20231

Sir:

Before the above-captioned application is taken up for examination, entry of the following amendment is respectfully requested (a marked up version pursuant to 37 C.F.R. 1.21 is attached hereto):

IN THE CLAIMS:

Please cancel claims 16, 19-21, 24-27, 29-32 without prejudice or disclaimer.

4. (Amended.) The variant of claim 1 or 3, wherein the parent cutinase:

a) is native to a filamentous fungus,

b) has an amino acid sequence which can be aligned with the cutinase of *H. insolens* strain DSM 1800, or

c) has an amino acid sequence which is at least 50 % homologous to the cutinase of *H. insolens* strain DSM 1800.

7. (Amended.) The variant of claim 1, which has one to twenty of such substitutions.

8. (Amended.) A variant of a parent fungal cutinase, which variant comprise a substitution of at least one amino acid residue corresponding to one or more of the following substitutions in the cutinase of *Humicola insolens* strain DSM 1800 (*H. insolens* cutinase numbering):

a) S48E +A88H +N91H +R189V

b) Q1L +L2K +G8D +N15D

c) N44D +A130V

d) Q1C +L2V +G120D

e) A88L +R189A

f) S48E +L66I +A88L +I169A +R189H

g) A88V +S116K +S119P +Q139R +I169V +R189V

h) A88V +R189A

i) S48K +A88H +I169G +R189H

j) Q1L +L2Q +A4V +S11T

k) T164S

l) L174F

m) H49Y

n) Q1L +L2K +G8D +N15D +S48E +A88H +N91H +R189V

o) Q1L +L2K +G8D +N15D +N44D +A130V

p) Q1L +L2K +G8D +N15D +S48E +A88H +N91H +A130V +R189V

q) G8D +N15D +A16T

r) A130V

s) Q1C +L2V

t) G8D +N15D +A16T

u) G8D +N15D +S48E +A88H +N91H +A130V +R189V

v) G8D +N15D +T29M +S48E +A88H +N91H +A130V +R189V

w) G8D +N15D +T29I +S48E +A88H +N91H +A130V +R189V and/or

x) G8D +N15D +T29C +S48E +A88H +N91H +A130V +R189V

y) G8D +N15D +S48E +A88H +N91H +A130V +L174F +I178V +R189V

z) G8D +N15D +S48E +A88H +N91H +A130V +T166M +I168F +R189V

aa) G8D +N15D +S48E +A88H +N91H +A130V +T166I +L167P +R189V

bb) G8D +N15D +V38H +S48E +A88H +N91H +A130V +I169T + R189V

cc) G8D +N15D +V38H +S48E +A88H +N91H +A130V +R189V

dd) G8D +N15D +T29M +S48E +A88H +N91H +A130V +T166I +L167P +R189V

9. (Amended.) The variant of claim 1, which further comprises at least one amino acid substitution at positions corresponding to Q1, L2, E6, E10, S11, A14, N15, F24, L46, E47, R51, D63, L138 and/or E179 (*H. insolens* cutinase numbering).

10. (Amended.) The variant of claim 1, further comprising at least one substitution corresponding to Q1P, L2V, E6Q, E10Q, S11C, A14P, N15T, F24Y, L46I, E47K, R51P, D63N, L138I and/or E179Q (*H. insolens* cutinase numbering).

11. (Amended.) The variant of claim 1, further comprising substitutions corresponding to E6Q +A14P +E47K +R51P +E179Q.

12. (Amended.) The variant of claim 1, which has hydrolytic activity towards terephthalic acid esters, particularly towards cyclic tri(ethylene terephthalate) and/or Terephthalic acid bis(2-hydroxyethyl)ester dibenzoate (BETEB).

13. (Amended.) The variant of claim 1, which has a denaturation temperature which is at least 5° higher than the parent cutinase, particularly measured at pH 8.5.

14. (Amended.) A DNA sequence encoding the variant of claim 1.

15. (Amended.) A vector comprising the DNA sequence of claim 14.

17. (Amended.) A method of producing the variant of claim 1 or 3, comprising:

a)cultivating a cell comprising a DNA sequence encoding the variant of claim 1 or 3 so as to express and optionally secrete the variant, and

b) recovering the variant.

22. (Amended.) The method of claim 18, wherein the parent cutinase:

a)is native to a filamentous fungus,

b) has an amino acid sequence which can be aligned with the cutinase of *H. insolens* strain DSM 1800, or

c) has an amino acid sequence which is at least 50 % homologous to the cutinase of *H. insolens* strain DSM 1800.

REMARKS

Claims 16, 19-21, 24-27, 29-32 are canceled in order to reduce the filing fee. Claims 1-15, 17, 18, 22, 23 and 28 are pending. Claims 4, 7-15, 17 and 22 have been amended, generally, to remove multiple dependency in order to reduce the filing fee. There is no new matter added, and entry of the amendment is respectfully requested.

This application contains a Sequence Listing. Applicants enclose a 3.5" floppy disk containing the Sequence Listing. The content of the attached paper entitled "SEQUENCE LISTING" and of the accompanying identically labelled diskette is the same.

The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

Date: June 1, 2001

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For: Cutinase Variants

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Sir:

Below is a marked-up version of the amendments made in the accompanying amendment.

IN THE CLAIMS:

4. (Amended.) The variant of [any preceding] claim 1 or 3, wherein the parent cutinase:

a)is native to a filamentous fungus[, particularly a strain of *Humicola* or *Fusarium*, particularly *H. insolens* or *F. solani pisi*, most particularly *H. insolens* strain DSM 1800],

b) has an amino acid sequence which can be aligned with the cutinase of *H. insolens* strain DSM 1800, or

c) has an amino acid sequence which is at least 50 % homologous to the cutinase of *H. insolens* strain DSM 1800[, particularly at least 70 % homologous, more particularly at least 80 % homologous].

7. (Amended.) The variant of [any preceding] claim 1, which has one to twenty of such substitutions[, particularly 2-15].

8. (Amended.) [The variant of any preceding claim] A variant of a parent fungal cutinase, which variant comprise a substitution of at least one amino acid residue corresponding to [comprising substitutions corresponding to] one or more of the following substitutions in the cutinase of *Humicola insolens* strain DSM 1800 (*H. insolens* cutinase numbering):

a)S48E +A88H +N91H +R189V

b) Q1L +L2K +G8D +N15D

c) N44D +A130V

d) Q1C +L2V +G120D

e) A88L +R189A

f) S48E +L66I +A88L +I169A +R189H

g) A88V +S116K +S119P +Q139R +I169V +R189V

h) A88V +R189A

i) S48K +A88H +I169G +R189H

j) Q1L +L2Q +A4V +S11T

k) T164S

l) L174F

m) H49Y

n) Q1L +L2K +G8D +N15D +S48E +A88H +N91H +R189V

o) Q1L +L2K +G8D +N15D +N44D +A130V

p) Q1L +L2K +G8D +N15D +S48E +A88H +N91H +A130V +R189V

q) G8D +N15D +A16T

r) A130V

s) Q1C +L2V

t) G8D +N15D +A16T

u) G8D +N15D +S48E +A88H +N91H +A130V +R189V

v) G8D +N15D +T29M +S48E +A88H +N91H +A130V +R189V

w) G8D +N15D +T29I +S48E +A88H +N91H +A130V +R189V and/or

x) G8D +N15D +T29C +S48E +A88H +N91H +A130V +R189V

y) G8D +N15D +S48E +A88H +N91H +A130V +L174F +I178V +R189V

z) G8D +N15D +S48E +A88H +N91H +A130V +T166M +I168F +R189V

aa) G8D +N15D +S48E +A88H +N91H +A130V +T166I +L167P +R189V

bb) G8D +N15D +V38H +S48E +A88H +N91H +A130V +I169T + R189V

cc) G8D +N15D +V38H +S48E +A88H +N91H +A130V +R189V

dd) G8D +N15D +T29M +S48E +A88H +N91H +A130V +T166I +L167P +R189V

9. (Amended.) The variant of [any preceding] claim 1, which further comprises at least one amino acid substitution at positions corresponding to Q1, L2, E6, E10, S11, A14, N15, F24, L46, E47, R51, D63, L138 and/or E179 (*H. insolens* cutinase numbering).

10. (Amended.) The variant of [the preceding] claim 1, further comprising at least one substitution corresponding to Q1P, L2V, E6Q, E10Q, S11C, A14P, N15T, F24Y, L46I, E47K, R51P, D63N, L138I and/or E179Q (*H. insolens* cutinase numbering).

11. (Amended.) The variant of [the preceding] claim 1, further comprising substitutions corresponding to E6Q +A14P +E47K +R51P +E179Q.

12. (Amended.) The variant of [any preceding] claim 1, which has hydrolytic activity towards terephthalic acid esters, particularly towards cyclic tri(ethylene terephthalate) and/or Terephthalic acid bis(2-hydroxyethyl)ester dibenzoate (BETEB).

13. (Amended.) The variant of [any preceding] claim 1, which has a denaturation temperature which is at least 5° higher than the parent cutinase, particularly measured at pH 8.5.

14. (Amended.) A DNA sequence encoding the variant of [any preceding] claim 1.

15. (Amended.) A vector comprising the DNA sequence of [the preceding] claim 14.

17. (Amended.) A method of producing the variant of [any of claims 1-13] claim 1 or 3, comprising:

a)cultivating [the cell of claim 16] a cell comprising a DNA sequence encoding the variant of claim 1 or 3 so as to express and optionally secrete the variant, and

b) recovering the variant.

22. (Amended.) The method of [any preceding claim] claim 18, wherein the parent cutinase:

a)is native to a filamentous fungus[, particularly a strain of *Humicola* or *Fusarium*, particularly *H. insolens* or *F. solani pisi*, most particularly *H. insolens* strain DSM 1800],

b) has an amino acid sequence which can be aligned with the cutinase of *H. insolens* strain DSM 1800, or

c) has an amino acid sequence which is at least 50 % homologous to the cutinase of *H. insolens* strain DSM 1800[, particularly at least 70 % homologous, more particularly at least 80 % homologous].